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*Published in:*  
Chemistry

*DOI:*  
[10.1002/chem.200801407](https://doi.org/10.1002/chem.200801407)

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*Document Version*  
Final author's version (accepted by publisher, after peer review)

*Publication date:*  
2008

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*Citation for published version (APA):*

Weiner, B., Poelarends, G. J., Janssen, D. B., & Feringa, B. L. (2008). Biocatalytic Enantioselective Synthesis of N-Substituted Aspartic Acids by Aspartate Ammonia Lyase. *Chemistry*, 14(32), 10094-10100. <https://doi.org/10.1002/chem.200801407>

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# **CHEMISTRY**

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## **A EUROPEAN JOURNAL**

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### Supporting Information

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# **Biocatalytic enantioselective synthesis of N-substituted aspartic acids by aspartate ammonia lyase**

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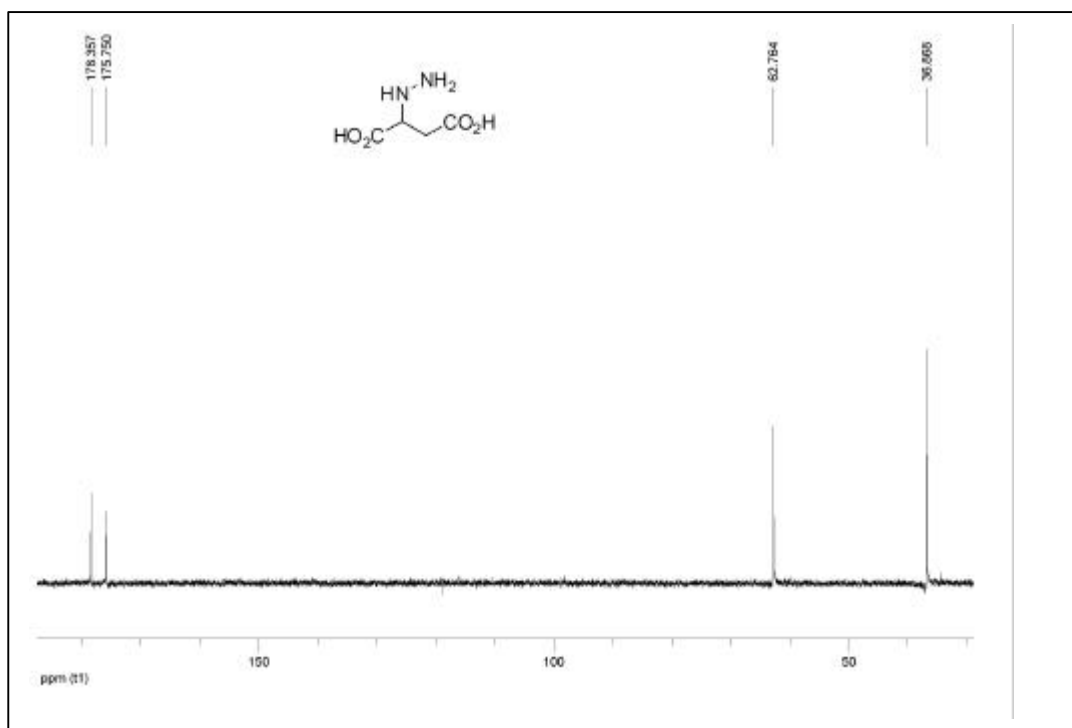
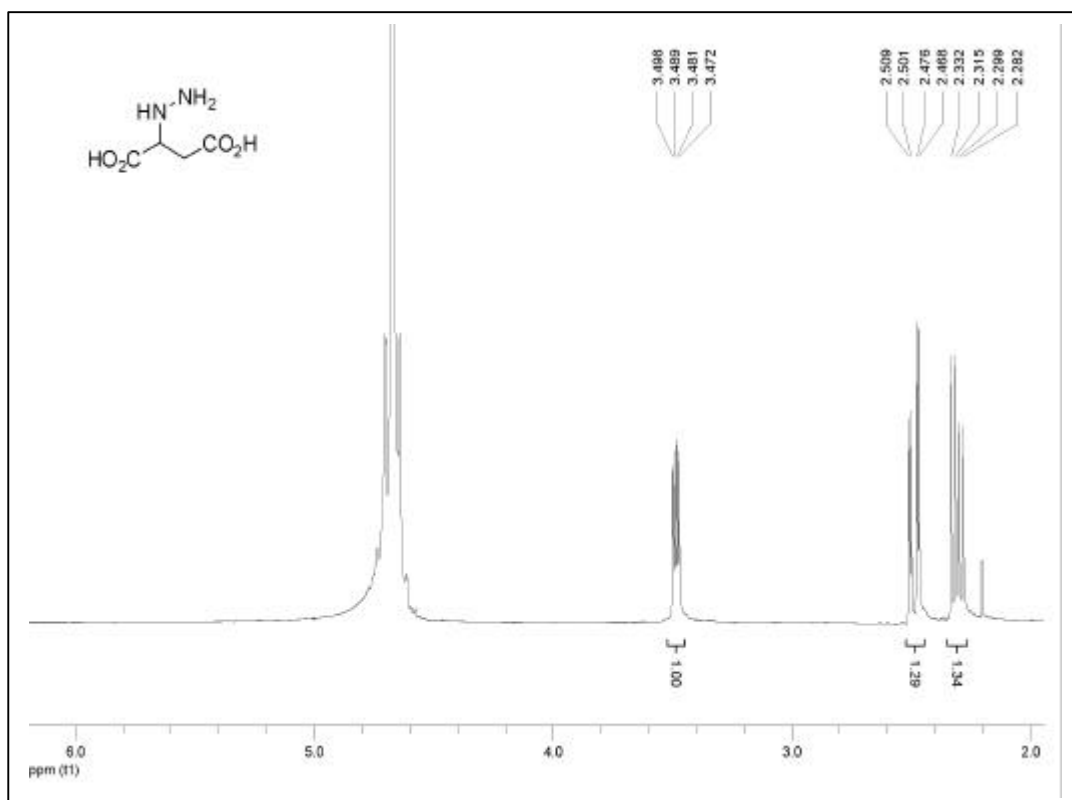
*[c] Department of Biochemistry, University of Groningen, Nijenborgh 4, 9747AG Groningen, The Netherlands.*

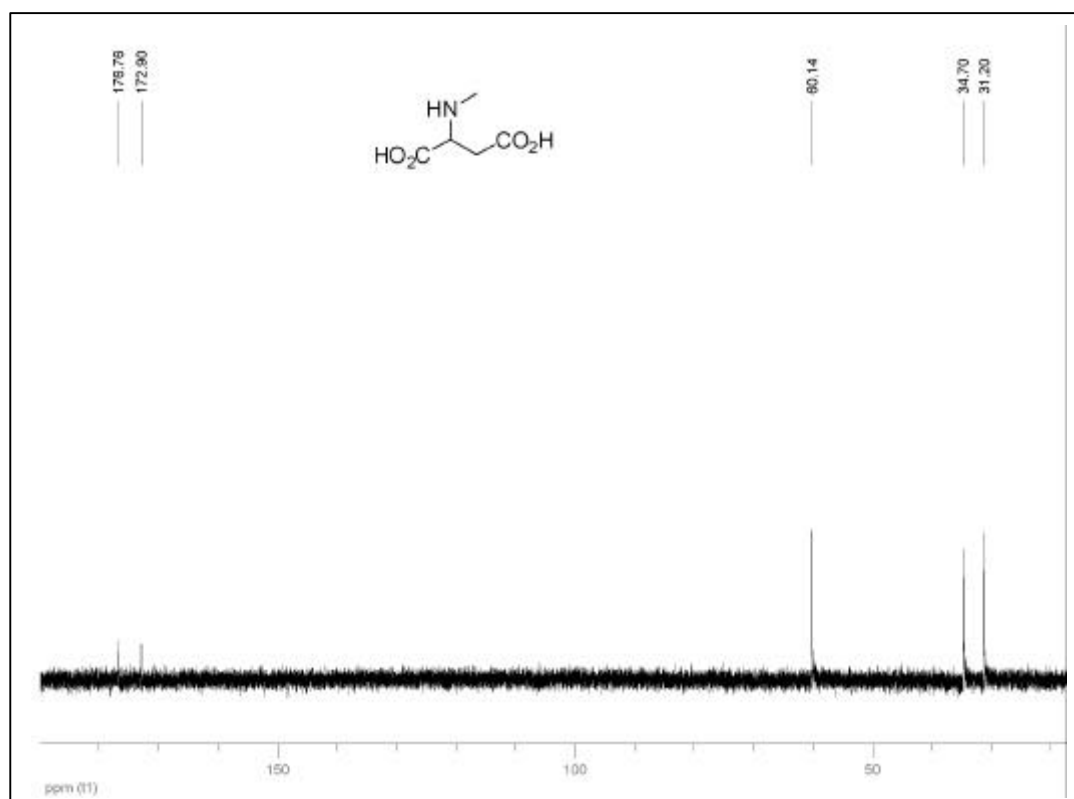
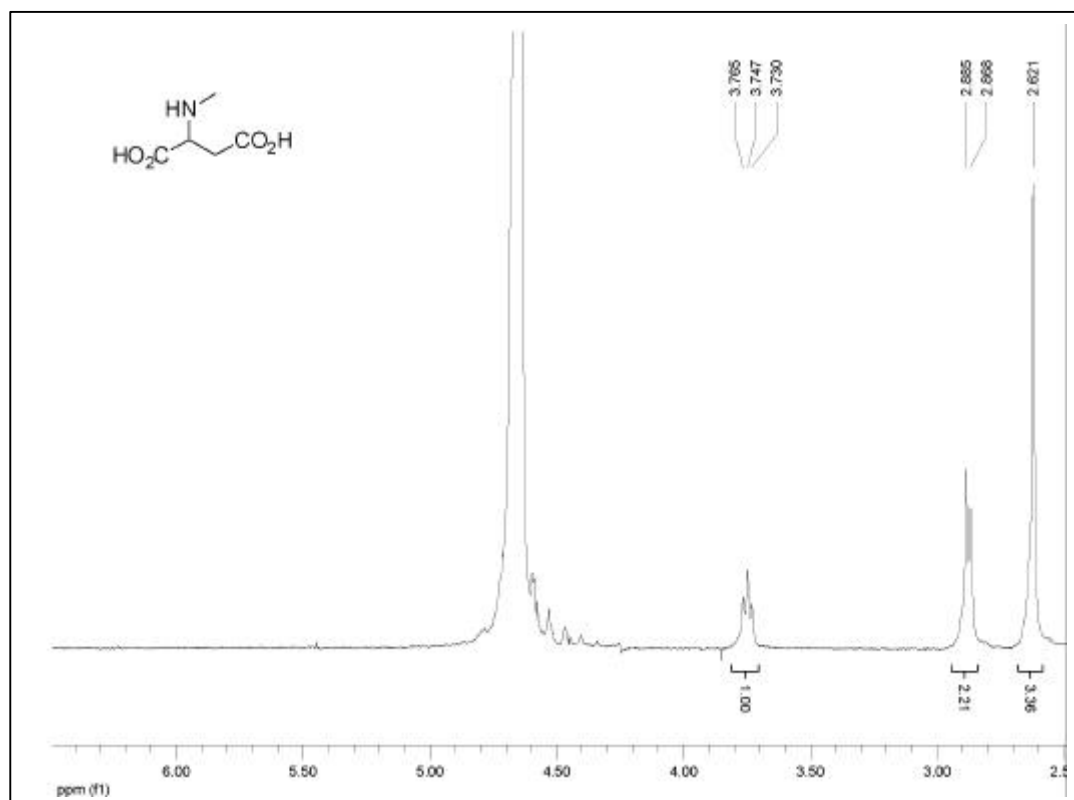
## General methods

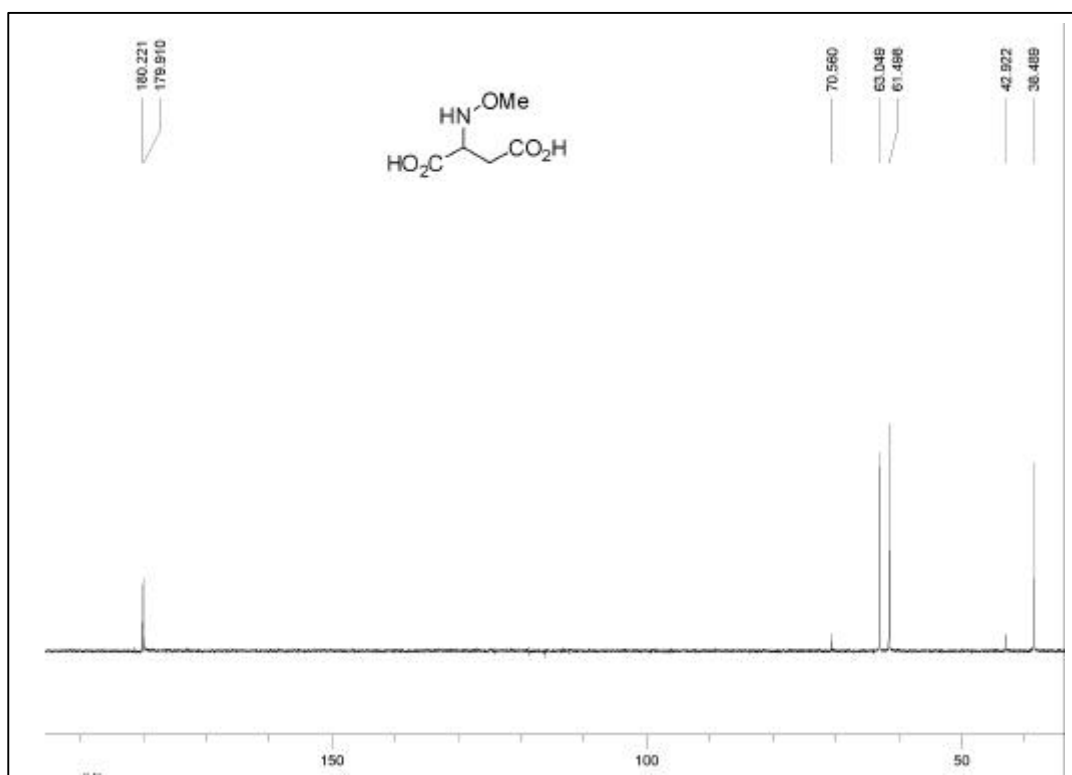
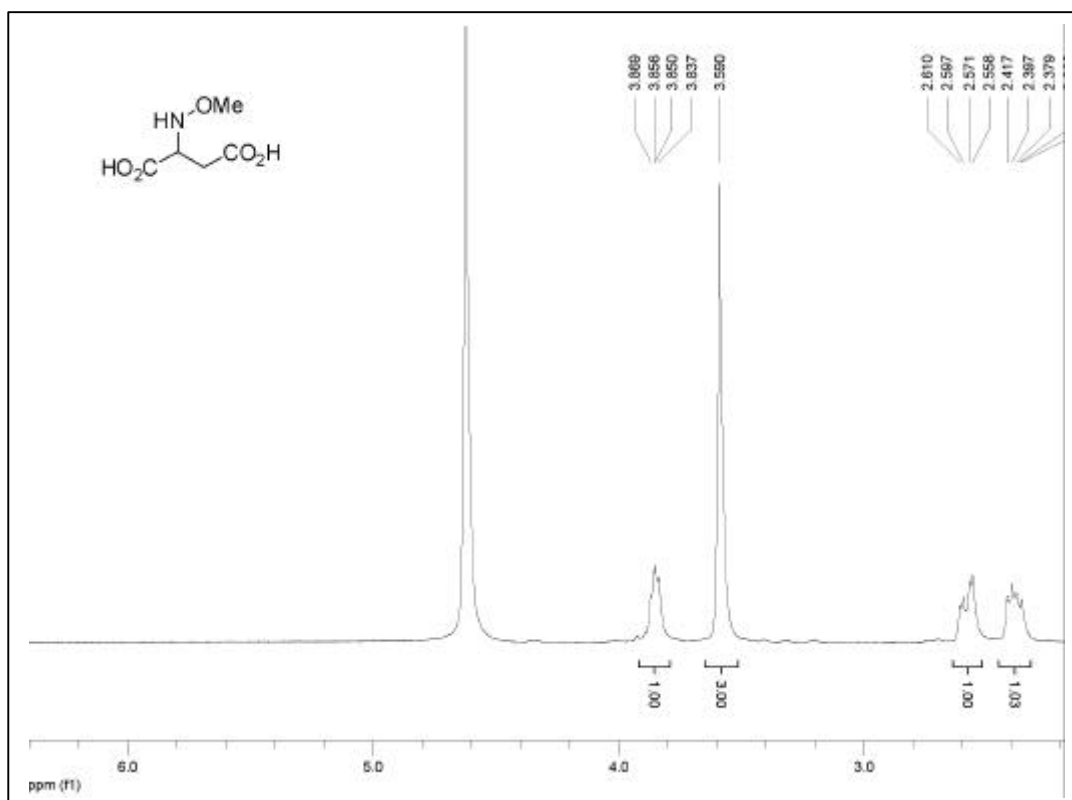
Reagents were purchased from Aldrich, Acros, Merck or Fluka and were used as provided, unless stated otherwise. All solvents were reagent grade. Enzymes used for the molecular biology procedures, DNA ladders, protein molecular weight standards, deoxynucleotide triphosphates (dNTPs), the high pure plasmid isolation kit, the high pure PCR product purification kit, and multipurpose agarose were purchased from F. Hoffmann-La Roche, Ltd. Oligonucleotides for DNA amplification and sequencing were synthesized by Sigma-Aldrich. All biocatalytic reactions were performed in 50 mL Greiner tubes, which were shaken at ~100 rpm in a waterbath at 37°C. Buffer and stock solutions of fumarate were prepared in distilled water and stored at 4°C. The pH of the solutions was adjusted with a Professional Meter PP-15 pH-meter from Sartorius. All moisture sensitive reactions were performed in round bottomed or modified Schlenk flasks, previously heated with a heatgun under oilpump vacuum, which were fitted with rubber septa under a positive pressure of nitrogen. Air- and moisture-sensitive liquids and solutions were transferred via syringe. Organic solutions were concentrated by rotary evaporation at 40–60°C. Lyophilization was performed with a ALPHA 2-4 LD plus freeze dryer from Christ. Flash column chromatography was performed as described by Still *et al.*<sup>[1]</sup> As stationary phase, Silia-P flash silica gel from Silicycle, size 40-63 µm, was used. For TLC analysis silica gel 60 from Merck (0.25 mm) impregnated with a fluorescent indicator (254 nm) was used. TLC plates were visualized by exposure to ninhydrin or phosphomolybdic acid (PMA) stain followed by brief heating with a heatgun. Ion exchange chromatography was performed with either Dowex 50 (H<sup>+</sup> form) activated with 1N HCl and rinsed with distilled water until a neutral pH was obtained (as assessed with pH indicator paper), or Amberlite IRA 140 (Cl<sup>-</sup> form) activated with 1N NaOH until chloride free and washed with distilled water until a neutral pH was obtained. SPE SCX columns were purchased from IST. Optical rotations were recorded with a Polartronic MH8 polarimeter from Schmidt + Haensch. The concentrations are given in g/100 mL. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on a Varian VXR-300 (300 MHz) or a Varian Mercury Plus (400 MHz) spectrometer. Chemical shifts for protons are reported in parts per million scale (δ scale) downfield from tetramethylsilane and are referenced to residual protium in the NMR solvents (CHCl<sub>3</sub>: δ = 7.25, H<sub>2</sub>O: δ = 4.67). Chemical shifts for carbon are calibrated to the middle signal of the <sup>13</sup>C-triplet of the solvent CDCl<sub>3</sub> (δ = 77.0). HPLC spectra were obtained using a Shimadzu LC-20AD equipped with a Chiralpak OD-H column. Reversed phase HPLC was performed on a Shimadzu LC-10AD VP using either a C6 Crownpack column or an Astec CLC-L column. Kinetic data were obtained on a Jasco V-550, V-560, or V-570 UV-spectrophotometer. Protein was analyzed by polyacrylamide gel electrophoresis (PAGE) under either denaturing conditions using sodium dodecyl sulfate (SDS) or native conditions on gels containing 12% polyacrylamide. The gels were stained with Coomassie brilliant blue. Protein concentrations were measured using the Waddell method.<sup>[2]</sup> DNA sequencing was performed by GATC Biotech.

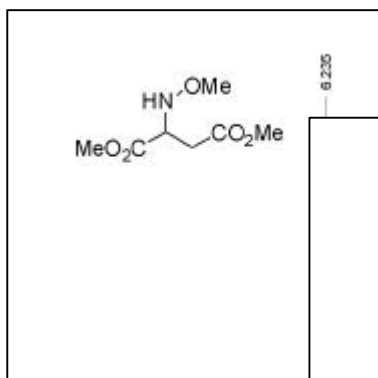
[1] W. C. Still, M. Kahn, A. Mitra, *J. Org. Chem.* **1978**, *43*, 2923-2925.

[2] W. J. Waddell, *J. Lab. Clin. Med.* **1956**, *48*, 311-314.



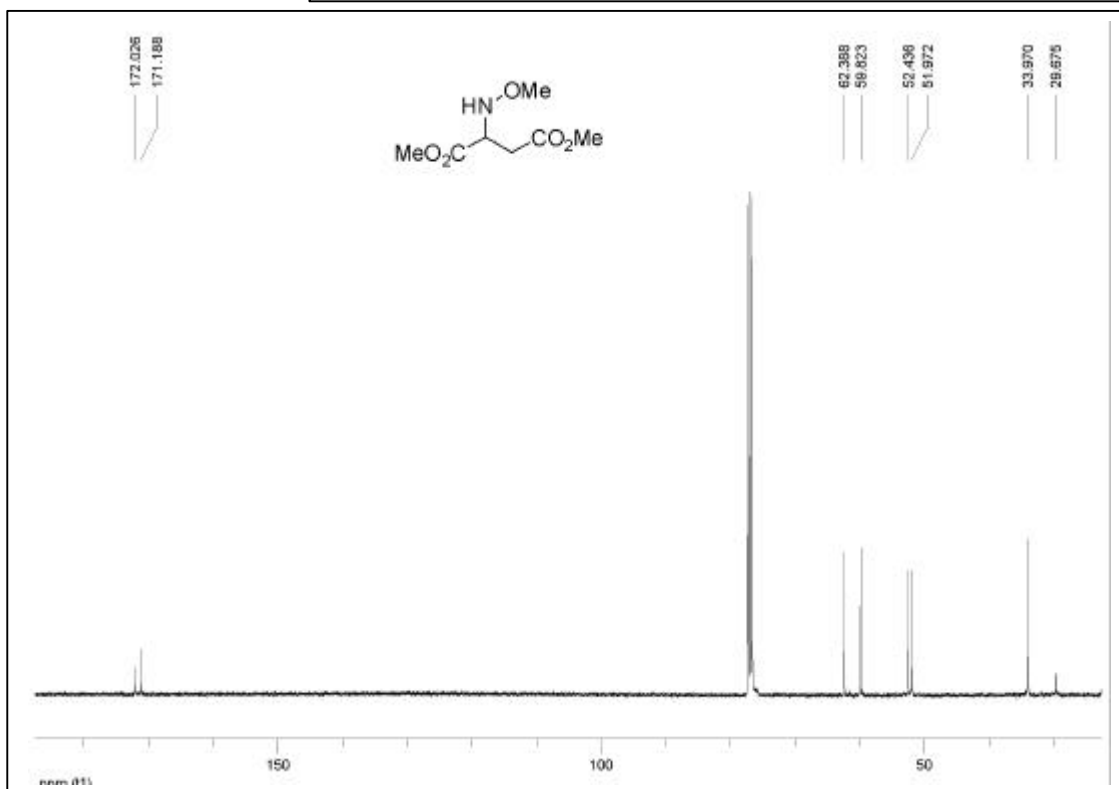
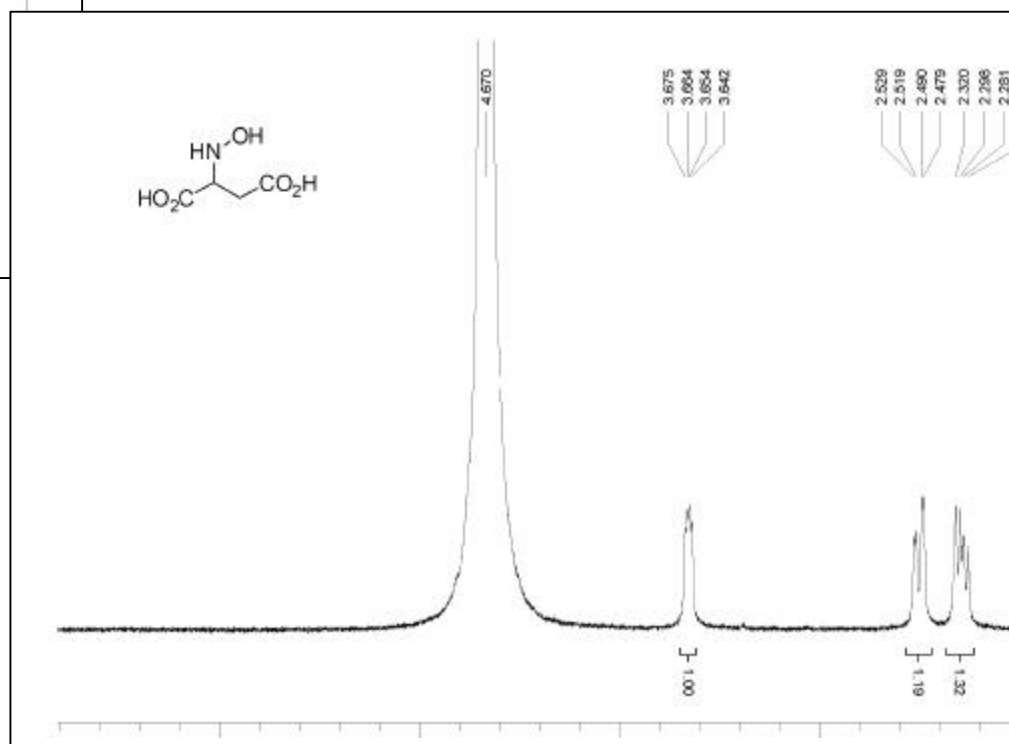




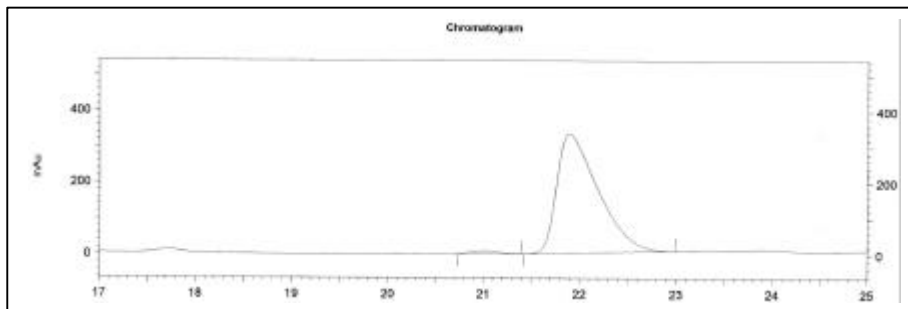
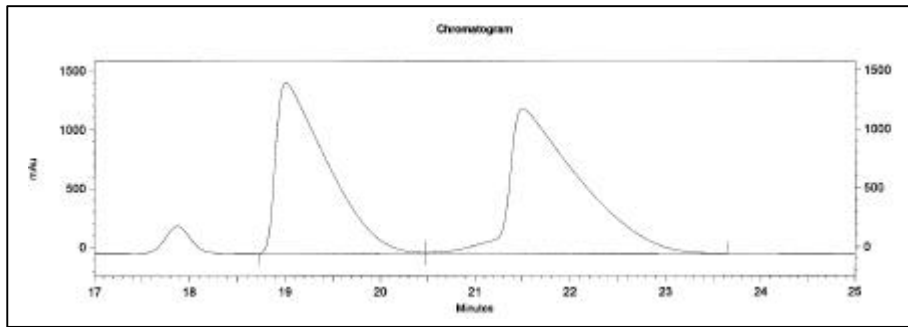


### HPLC analysis

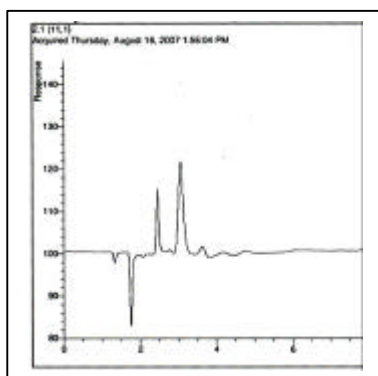
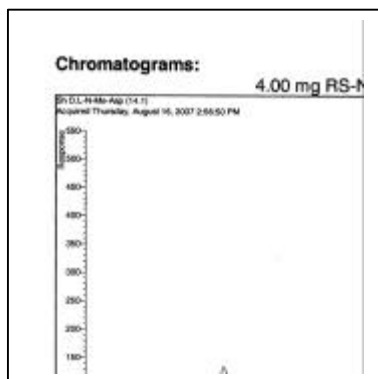
*N*-methoxyaspartic acid dimethylester





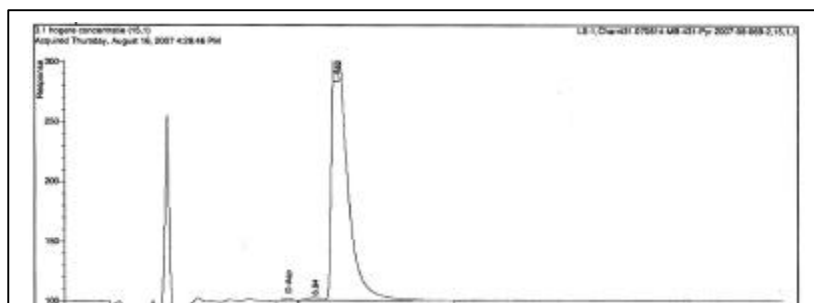
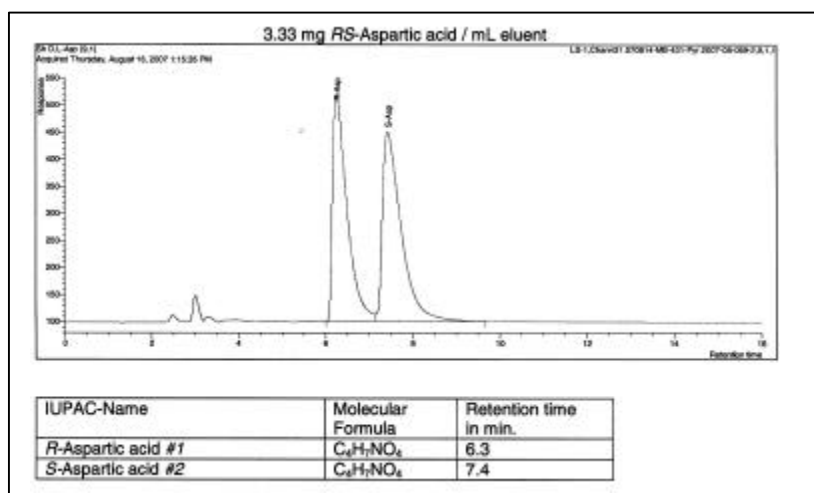


***N*-methylasspartic acid**



### L-Aspartic acid from 2-hydrazinosuccinic acid

The crude 2-hydrazinosuccinic acid (5.0 mmol) was dissolved in H<sub>2</sub>O (12 mL), and a catalytic amount of PtO<sub>2</sub> followed by 3 drops of concentrated acetic acid were added. A hydrogen balloon was placed on top of the Schlenk flask and the solution stirred vigorously over night. The mixture was filtered over celite and the filtrate was evaporated. The crude aspartic acid was purified by IEC on cationic Dowex 50 (H<sup>+</sup>, 20-50 mesh, washed with water) by elution with 5% NH<sub>3</sub>-solution and lyophilized. <sup>1</sup>H NMR (300 MHz; D<sub>2</sub>O)  $\delta$  = 2.55 (dd, <sup>2</sup>*J* (2,2) = 17.4 Hz, <sup>3</sup>*J* (2,1) = 8.7 Hz, 1H; CH<sub>2</sub>), 2.69 (dd, <sup>2</sup>*J* (2,2) = 17.5 Hz, <sup>3</sup>*J* (2,1) = 4.1 Hz, 1H; CH<sub>2</sub>), 3.76 (dd, <sup>3</sup>*J* (1,2) = 9.0 Hz, <sup>3</sup>*J* (1,2) = 3.6 Hz, 1H; CH); HPLC (Astec CLC-L, 2mM CuSO<sub>4</sub> in H<sub>2</sub>O : MeOH 90 : 10, flow 1.0 mL/min, 40°C) 6.3 min (D-Asp), 7.4 min (L-Asp), 99.5% *ee*.



### L-Aspartic acid from N-hydroxyaspartic acid

The crude N-hydroxyaspartic acid (1.0 mmol) was dissolved in H<sub>2</sub>O (5 mL), and a catalytic amount of PtO<sub>2</sub> followed by 5 drops of concentrated acetic acid were added. A hydrogen balloon was placed on top of the Schlenck flask and the solution stirred vigorously over night. The mixture was filtered over celite and the filtrate was evaporated. The crude aspartic acid was purified *via* IEC on cationic Dowex 50 (H<sup>+</sup>, 20-50 mesh, washed with water) by elution with 5% NH<sub>3</sub>-solution, and lyophilized (0.16 g, 0.80 mmol, 80%). HPLC (C6 Crownpack, HClO<sub>4</sub>, pH 2.0, flow 0.3 mL/min, 0°C) 4.33 min (D-Asp), 6.23 (L-Asp), 97% *ee*)

